

OLIGONUCLEOTIDE FOR GENOTYPING *MYCOPLASMA* AND ITS
RELATED STRAINS, MICROARRAY COMPRISING THE
OLIGONUCLEOTIDE, AND METHOD FOR DETECTING STRAINS
USING THE MICROARRAY

5

Technical Field

The present invention relates to a method for detecting
Mycoplasma and its related strains which are a source of contamination of
cell lines and biological products and human pathogens. More particularly,
10 the present invention relates to genus-specific and species-specific
oligonucleotides for genotyping *Mycoplasma*, *Acholeplasm* and
Ureaplasma strains, a microarray comprising the oligonucleotides, and a
method for detecting strains using the microarray

15 **Background Art**

Mycoplasma is a prokaryote pertaining to Mollicute family without
cell wall, which was known as a hospital acquired pathogen causing
pneumonia via infection of genital and respiratory organs of human as
well as livestock such as pig and cow. Recently, *Mycoplasma* is more
20 seriously understood as a major contaminant of cell culture and cell line

Especially, as the development and production of biological
products for protecting and treating human diseases increases, the
contamination of various pathogens provided by microorganism or
clinical sample in the process of production became a serious problem.
25 Examples of the biological products are an oncolytic virus, vaccine, a
gene therapy vector and a recombinant protein. They have been found
to be contaminated by bacteria, fungus, virus, *Mycoplasma* and its
related strains (Doblhoff-Dier et al., 2001). The reason of the
contamination is an organism contaminated in media components or
30 experimental instruments and cross-contamination of microorganism and
virus in air (Jung et al., 2003). Also, the contamination can be occurred

by a cross-contamination of already-infected WCB (Working Cell Bank) which is used for mass production of biological products (Wisher et al., 2002).

It is reported that, among these contamination sources, about
5 15~35% of cell culture or cell line is infected by *Mycoplasma* and its related strains (Hopert et al., 1993). This also makes experimental results incredible because it can change characteristics of cells such as abnormal synthesis of DNA, RNA and protein by binding to host cell wall (Kong et al., 2001). As gene therapy and cell therapy are getting into
10 the spotlight recently, an assay for infection of stem cell and cord blood by *Mycoplasma* and its related strain became more important. Therefore, for the credible and reproducible experimental results and the quality control of commercialized biological products, it is essential to detect an infection with Mycoplasma and its related strains.

Under this situation, Europe community make it a rule that, for
15 credibility of safety and quality of food and drug, GMP (Good Manufacturing Practice) and QC (Quality Control) should be submitted and cell banks such as MCB (Master Cell Bank) and WCB should be subjected to an assay for detection of virus, fungus and bacteria such as
20 *Mycoplasma* (Doblhoff-Dier et al., 2001).

About 100 kinds of bacteria pertaining to Mollicute family without cell wall have been found so far, including *Acholeplasma*, *Enteroplasma*, *Mesoplasma*, *Mycoplasma*, *Ureaplasma* and *Spiroplasma*. Among them, about 20 kinds of *Mycoplasma*, *Acholeplasma* and *Ureaplasma*
25 are major contamination source of cell culture. These are referred to as "Mycoplasma and its related strains" in this specification. About 95% of the contaminants are covered by *M. arginini*, *M. fermentans*, *M. orale*, *M. hyorhina*, *M. hominis*, *M. salivarium*, *M. pirum*, *A. laidlawii* (Dorigo-zetsma et al., 1997). However, *Mycoplasma* is difficult to be cultured in
30 extracellular media and turbidity is rare in the culture. Therefore, there has been a need to the rapid and accurate genotypic detection method

which can trace a contamination source of *Mycoplasma* and its related strains.

Conventional *Mycoplasma* detection methods are the culturing method, the DNA fluorochoime stain method, the immunofluorescence method, and the polymerase chain reaction (PCR) method (Dorigo-
5 zetsma et al., 1997). However, the culturing method has a drawback that extracellular culturing is difficult, preparing its media is complex by adding supplements such as serum and culturing time is too long, about 4 days ~ 3 weeks according to the kinds of strains (Jensen et al., 2003).
10 The DNA fluorochoime stain method such as Hoechst 33258 stain has a drawback that culturing condition is too difficult to match and subjective inspectors can make a misjudgment (Chen et al., 1997). The immunofluorescence method such as ELISA has a drawback that bacteria having similar antigen with *Mycoplasma* such as *Streptococcus*
15 *milleri* group and *Staphylococcus aureus* may raise a false positive signal due to of low specificity (Hopert et al., 1993). The PCR method makes use of 16S/23S intergenic spacer region (ITS) and a gene coding 169 kDa of P1 cyadhesion proteine which represent variety of *Mycoplasma* (Uphoff et al., 2002). The P1 gene, a surface antigen
20 gene, has several subtypes representing diversity and has been used as a target gene for serological detection using immune reaction and genotypic detection using restriction fragment length polymorphism (RFLP) to identify *Mycoplasma* (Campo et al., 1998). However, most of conventional PCR methods use a primer designed based on 16S rRNA
25 which is a common sequence of prokaryotes, and second PCR or nested PCR having high sensitivity can make a cross-contamination of *Mycoplasma* dispersed in air and an amplification of a bacteria similar with *Mycoplasma* in classification (Uphoff et al., 2002).

To overcome the above limitations of the conventional detection
30 methods, a genotypic detection method using probes have been developed recently, which make it possible to analyze many kinds of

genes in a short time using DNA hybridization principle based on gene sequencing and detect specifically a single base change using a proper hybridization condition between specific probe and target DNA.

5 The present inventors developed ITS-derived oligonucleotides capable of detecting *Mycoplasma* and its related strains, which are important in genotypic detection, and a microarray comprising the oligonucleotides as a probe for detecting *Mycoplasma* and its related strains.

10 **Disclosure of the Invention**

It is a first object of the present invention to provide oligonucleotides for detecting *Mycoplasma* and its related strains designed based on their ITS base sequences.

15 It is another object of the present invention to provide novel ITS sequences of *Mycoplasma bovis*, *Mycoplasma cloacale*, *Mycoplasma falconis*, *Mycoplasma faucium*, *Mycoplasma spermatophilum* and *Mycoplasma synoviae*, which is useful for detecting *Mycoplasma* and its related strains.

20 It is another object of the present invention to provide a microarray comprising genus-specific and species-specific oligonucleotides for detecting *Mycoplasma* and its related strains as probes.

It is another object of the present invention to provide a method for detecting *Mycoplasma* and its related strains using the microarray.

25 It is another object of the present invention to provide a kit for diagnosing *Mycoplasma* and its related species infection individually or simultaneously, comprising genus-specific and species-specific oligonucleotides for genotyping *Acholeplasma* and related strains.

30 According to an aspect of the present invention, there is provided a purified ITS (internal transcribed spacer) target DNA for genotyping

Mycoplasma strains, comprising any one sequence selected from SEQ ID Nos. 1 to 6.

SIQ ID Nos. 1 to 6 are base sequences of ITS (internal transcribed spacer) of *Mycoplasma bovis*, *Mycoplasma cloacale*,
5 *Mycoplasma falconis*, *Mycoplasma faucium*, *Mycoplasma spermatophilum* and *Mycoplasma synoviae*, which was newly obtained by base sequencing analysis.

The ITS target DNA of the present invention can be used indirectly for designing probes or primers used for genotyping
10 *Mycoplasma* strains or directly for genotyping *Mycoplasma* strains via PCR amplification.

According to another aspect of the present invention, there is provided an oligonucleotide for genus-specific genotyping of
15 *Mycoplasma* and *Ureaplasma* strains, comprising any one sequence selected from SEQ ID Nos. 7 to 21 or its complementary sequence.

According to another aspect of the present invention, there is provided an oligonucleotide for genus-specific genotyping of
Acholeplasma strains, comprising any one sequence selected from SEQ ID Nos. 22 to 27 or its complementary sequence.

20 According to another aspect of the present invention, there is provided an oligonucleotide for species-specific genotyping of *Mycoplasma* and *Ureaplasma* strains, comprising any one sequence selected from SEQ ID Nos. 28 to 127 or its complementary sequence.

25 According to another aspect of the present invention, there is provided an oligonucleotide for species-specific genotyping of *Acholeplasma* strains, comprising any one sequence selected from SEQ ID Nos. 128 to 133 or its complementary sequence.

The oligonucleotides according to the present invention are designed based on multiple sequence alignment of ITS (internal
30 transcribed spacer) sequences, which are present between 16S rRNA and 23S rRNA of *Mycoplasma* and its related species. The

oligonucleotides can be used as primers for PCR amplification in order to genotype *Mycoplasma* and its related species or as probes for hybridization reaction in order to genotype *Mycoplasma* and its related species.

5 According to another aspect of the present invention, there is provided a microarray comprising more than one oligonucleotides selected from genus-specific and species-specific oligonucleotides for genotyping *Mycoplasma*, *Acholeplasma* and *Ureaplasma* strains according to any one from claims 2 to 5 as probes attached on a support.

10 In the microarray according to the present invention, the probes may be any materials having base sequence, preferably any one selected from a group consisting of DNA (Deoxyribose Nucleic acid), RNA (Ribose Nucleic Acid), and nucleic acid analogues such as PNA (Peptide Nucleic Acid), LNA (Locked Nucleic Acid) and HNA (Hexitol
15 Nucleic Acid).

 In the microarray according to the present invention, the support may be any materials to which the probes can be attached, preferably any one selected from a group consisting of slide glass, plastic, membrane, semiconductive chip, silicon and gel. The microarray
20 according to the present invention can be manufactured using conventional method such as pin microarray, ink jet, photolithography or electric array method.

 The microarray according to the present invention can be used for simultaneously genotyping various *Mycoplasma* and its related
25 species which are known as a major contaminant of biological drug and cell line as well as a human pathogen from one sample, as the microarray comprises genus-specific and species-specific oligonucleotides for genotyping *Mycoplasma* and its related species as a set attached a support

30 According to another aspect of the present invention, there is provided a method for detecting *Mycoplasma*, *Acholeplasma* and

Ureaplasma strains, comprising the following steps:

- a) extracting nucleic acids from a sample;
- b) amplifying target DNA among the extracted nucleic acids;
- c) hybridizing the amplified target DNA with probes of the
- 5 microarray according to the above present invention; and
- d) detecting signals generated from the hybridization reaction.

In the detection method according to the present invention, the sample may be biological drug, cell line, or human tissues or serum. The purifying step can be performed using conventional DNA or RNA
10 purification method or kit. The signal detecting step can be performed using a conventional fluorescence scanner after binding conventional fluorescent dyes such as Cy5 or Cy3.

According to another aspect of the present invention, there is provided a kit for diagnosing *Mycoplasma* and its related species
15 infection, comprising more than one oligonucleotide selected from genus-specific and species-specific oligonucleotides for genotyping *Acholeplasma*, *Mycoplasma* and *Ureaplasma* strains according to the above present invention.

In the kit according to the present invention, the oligonucleotides
20 are used as probes for hybridizing with target sample and may be contained in a proper vessel. The probes may be labeled with a radioactive or non-radioactive labeling agent, the latter comprises conventional biotin, Dig(digoxigenin), FRET(fluorescence resonance energy transfer) or fluorescent dye (Cy5 or Cy3). Further, the
25 oligonucleotides can be used as primers for PCR amplification. In this case, the kit may contain DNA polymerase, 4 dNTPs and PCR buffer for PCR reaction. In addition, the oligonucleotides can be attached to a microarray as probes. In this case, the kit may contain hybridization reaction buffer, PCR kit containing primers for amplifying a target gene,
30 washing solution for the unhybridized DNA, dyes, washing solution for unbound dyes and manual sheet for the microarray.

Hereafter, the present invention will be described in more detail.

The present invention provides a method for detecting or genotyping *Mycoplasma* and its related strains which is a major contamination source of cell lines and biological products and a human pathogen, comprising the following steps:

a) if necessary, extracting nucleic acids from a sample such as cell lines, biological products or human tissue or serum;

b) if necessary, amplifying target DNA of *Acholeplasma*, *Mycoplasma* and *Ureaplasma* strains among the extracted nucleic acids using more than one proper primers;

c) hybridizing the amplified target DNA with probes having a sense or antisense or complementary sequences of genus-specific and species-specific oligonucleotides of *Acholeplasma*, *Mycoplasma* and *Ureaplasma* strains disclosed in Tables 2 and 3; and

d) detecting signals generated from the hybridization reaction.

From the detected signals in the step d), the existence of *Mycoplasma* and its related strains in the sample can be predicted.

The present inventors carried out a sequence analysis of ITS regions of many *Acholeplasma*, *Mycoplasma* and *Ureaplasma* strains to obtain genus-specific and species-specific oligonucleotides for detecting *Mycoplasma* and its related stains which can be a basis of developing a specific and sensitive hybridization assay. Also, the present inventors newly analyzed ITS sequences of newly found 6 *Mycoplasma* strains, which makes it possible to design probes capable of detecting more various *Mycoplasma* and its related strains.

Table 1 discloses ITS sequences of newly analyzed 6 strains among target sequences for detecting *Mycoplasma* strains, which correspond to SEQ ID Nos. 1 to 6. In the present invention, the probes for detecting *Mycoplasma* strains were designed based on the multiple alignment of ITS sequences of *Mycoplasma*.

FIGS. 1 and 2 show multiple sequence alignments of ITS regions

of *Mycoplasma*, *Acholeplasma* and *Ureaplasma* for selecting genus-specific and species-specific probes of *Mycoplasma* and its related strains. Genus-specific oligonucleotides of *Mycoplasma* and *Ureaplasma* were designed from conservative sequence region indicated by a box in FIGS. 1a to 1f. Species-specific oligonucleotides of *Mycoplasma* and *Ureaplasma* were designed from polymorphic sequence region outside the box in FIGS. 1a to 1f. Genus-specific oligonucleotides of *Acholeplasma* were designed from conservative sequence region indicated by a box in FIGS. 2a to 2c. Species-specific oligonucleotides of *Acholeplasma* were designed from polymorphic sequence region outside the box in FIGS. 2a to 2c.

In step b) of the present invention, the target DNA of *Acholeplasma*, *Mycoplasma* and *Ureaplasma* strains were amplified using more than one pair of proper primers. FIG. 3 shows PCR amplification of ITS target sequences of *Mycoplasma* and its related strains using a primer pair, MP16SF-2 and MP23SR-2. In FIG. 3, 1 is a PCR product of *M. arginini*, 2 is a PCR product of *M. arthritis*, 3 is a PCR product of *M. fermentans*, 4 is a PCR product of *M. hominis*, 5 is a PCR product of *M. hyorhina*, 6 is a PCR product of *M. neurolyticum*, 7 is a PCR product of *M. opalescens*, 8 is a PCR product of *M. orale*, 9 is a PCR product of *M. pirum*, 10 is a PCR product of *M. penetrans*, 11 is a PCR product of *M. pulmonis*, 12 is a PCR product of *M. salivarium*, 13 is a PCR product of *M. cloacale*, 14 is a PCR product of *M. falconis*, 15 is a PCR product of *M. faucium*, 16 is a PCR product of *M. hyosynoviae*, 17 is a PCR product of *M. muris*, 18 is a PCR product of *M. primate*, 19 is a PCR product of *M. spermatophilum*, 20 is a PCR product of *M. synoviae*, 21 is a PCR product of *M. pneumoniae*, 22 is a PCR product of *M. genitalium*, 23 is a PCR product of *M. bovis*, 24 is a PCR product of *U. urealyticum*, 25 is a PCR product of *A. laidlawii*.

In step c) of the present invention, the amplified target DNA were hybridized with probes for detecting *Mycoplasma* and its related strains.

Preferably, the probes may be a combination of more than one probes capable of simultaneously detecting many *Mycoplasma* and its related strains from a single sample. Practically, the probes are optimized to simultaneously hybridize with multiple target DNAs of *Mycoplasma* and its related strains under the same hybridization and washing conditions.

The present invention provides a microarray comprising a set of probes for detecting *Mycoplasma* and its related strains, which can simultaneously detect many *Mycoplasma* and its related strains from a single sample with a single experiment.

In the present invention, the term 'probe' means a single-stranded oligonucleotide having a sequence complementary to target DNA of *Mycoplasma*, *Acholeplasma* and *Ureaplasma*. The probe may have a sense, antisense or complementary sequence of SEQ ID Nos. disclosed in this specification as long as it can hybridize with one of double strands of target DNA. The oligonucleotide may be ribonucleotide (RNA), deoxynucleotide (DNA), peptide nucleic acid (PNA) or locked nucleic acid (LNA), and contain modified nucleotides such as Inosine only if it does not change their hybridization characteristics. Preferably, the genus-specific oligonucleotides for detecting *Mycoplasma*, *Acholeplasma* and *Ureaplasma* may have a base sequence of SEQ ID Nos. 7 to 27. Preferably, the species-specific oligonucleotides for detecting *Mycoplasma*, *Acholeplasma* and *Ureaplasma* may have a base sequence of SEQ ID Nos. 28 to 133.

FIG. 4 shows a microarray comprising probes for detecting genotypes of *Mycoplasma* and its related strains as a set on a support. In FIG. 4, each species name and SEQ IN Nos. are described which correspond to individual probes. The terms 'MP-C' and 'AP-C' mean *Mycoplasma* and *Ureaplasma* genus and *Acholeplasma* genus. FIG. 4 is no more than an example of probe compartment of the present invention, so compartment and layout of each probe can be varied.

In the present invention, newly analyzed ITS sequences of 6

Mycoplasma strains as a target DNA for detecting *Mycoplasma* and its related strains are as shown in Table 1. The genus-specific oligonucleotides for detecting *Mycoplasma*, *Acholeplasma* and *Ureaplasma* used in the present invention are as shown in Table 2. The species-specific oligonucleotides for detecting *Mycoplasma*, *Acholeplasma* and *Ureaplasma* used in the present invention are as shown in Table 3.

【Table 1】

Species	Sequence (5' → 3')	SEQ ID NO.
<i>M.bovis</i>	TTCTACGGAGTACACTTGTC TTTTATCACTATAAAAAAAGACTTATAACCAAAAT TACTAGACC TATATTTATTTATAAACGTCATGGC TTTTATTAATAGG TCAAAAGC TA TA TATCTAG TTTTIGAGAGAACATTC TC TCA TATG TTTTIGAAAAAC TGAATAGTAAA ATATTTTTCGATATTTACAACGACATCAAAAA TCAAAATTAATGG TTAATTGG TTTTIG ATTTCATCG AG TAAG TCA TATTTAATA TGA TTCA TTGAAA TGTCTTTAAAA TACACATC TAAACTAAC AACAATAGGAAAA TACTAC TTTTAAAA TAAGGAAGAG TTTTGGTGG ATGC	1
<i>M.colocals</i>	CTTC TACGGAGTACAATTC TCACTG TTTATGG AATTAAATTTG TATCCAG TTTTIGAG GAAC TTTCTCTCAATTTTGTTC TTTGAAAAAC TGAATATAGACATTGAAA TCAATAAA TTAATATTTCAAA TGT TTAGA TCAACC TATAGAA TATICAAGACATATACAAAAATA GGTCACTATTATTTTATAAATACT	2
<i>M.falconis</i>	CTTTC TACGGAGTACAAC TTC TGT TATGGAA TAA TATTGTATCCAG TTTTIGAGAGT ACTAATCTCT TTTTGT TCT TTTGAAAAAC TGAATATCGACATTGAAAAA TTAATAAT AATATTTCAAA GTTTTGA TCAACC TATAGAA TACAAAAATATAGACAACAATAGGT CATACAACAACATAACAAAACACT	3
<i>M.ferusium</i>	GAA TGTG TGGCTTCGAGACTAAAAG TTA TGGAAAAACATCG TATCCAG TTTTIGAG GAAC TAAAC TTC TC TTTTGT TCT TTTGAAAAAC TGAATA TAGACA TTGAAAAATAAA AAATTAATATTTCAAAGTTTGA TCAACC TATAGAA TACAAAA TCAATACAATAGG TCAATAC TATACAATTGCA TAAACAAAAA TACTATTAACAAGATAAGAG TTTTGTG GTGGATGCAATTGTAT	4
<i>M.sparmatophilum</i>	GTGGGGATGGATCACCTCC TTTCTACGGAGTACAACA TACATTCAAATTTGACT GAA TGT TATTAACCTTATTTTTCAC TAGGCC TTTTAAATA TATTTGT TTA TGTGACT TTTATGGCC TAAAGTCTTATATCTAGTTTGTAGAGGACATCC TCTCTAATTGT TCT TTGAAAAC TGAATAGTAAATTTTGTATATTTAC AACGACATC TAAATAATTGAAIT AAGTCAATTTG TTTAGATTCA TCGAGATAG TCA TTTTAAAAAATGATTCATTGAA ATG TCTTAAAAATACACATCAAAACAAACATCTATACAATAGGAA TTTATATACT TCTTACG GAGTACATTAATTTTACAAAAGGCATTTTATTAAC TGAAGCTTTTGTG AGAAAAATTTCTAAAAGCGG TGTGTATCGCTTTTGTGCTTGAGGCTATGTATTTA GTTTGTAGAGAACACCTCTCT TAAAAATGTCT TTTGAAAAAC TAAATAG TAATAAA GATATTTACACGACATCAAAAA TATAAA TTAATTAAGGTAA TTTGT TTTGTATCCG AG TTTAAATTA TGTAAATAA TTTATTAATA TGCTTTGAAATACATCA TAAACAATA TAACAATAGGACATATGTATCTAAC TTTTAAAAAAGT	5
<i>M.synoviae</i>	GTGGGGATGGATCACCTCC TTTCTACGGAGTACAACA TACATTCAAATTTGACT GAA TGT TATTAACCTTATTTTTCAC TAGGCC TTTTAAATA TATTTGT TTA TGTGACT TTTATGGCC TAAAGTCTTATATCTAGTTTGTAGAGGACATCC TCTCTAATTGT TCT TTGAAAAC TGAATAGTAAATTTTGTATATTTAC AACGACATC TAAATAATTGAAIT AAGTCAATTTG TTTAGATTCA TCGAGATAG TCA TTTTAAAAAATGATTCATTGAA ATG TCTTAAAAATACACATCAAAACAAACATCTATACAATAGGAA TTTATATACT TCTTACG GAGTACATTAATTTTACAAAAGGCATTTTATTAAC TGAAGCTTTTGTG AGAAAAATTTCTAAAAGCGG TGTGTATCGCTTTTGTGCTTGAGGCTATGTATTTA GTTTGTAGAGAACACCTCTCT TAAAAATGTCT TTTGAAAAAC TAAATAG TAATAAA GATATTTACACGACATCAAAAA TATAAA TTAATTAAGGTAA TTTGT TTTGTATCCG AG TTTAAATTA TGTAAATAA TTTATTAATA TGCTTTGAAATACATCA TAAACAATA TAACAATAGGACATATGTATCTAAC TTTTAAAAAAGT	6

[Table 2]

Genus	Probe	Sequence	SEQ ID NO.
<i>Mycoplasma</i>	MP-CP1	TTCTTTGAAAAC TGA	7
	MP-CP2	RWTC TTTVAAAAC TTTATWN	8
<i>M. arginini</i> , <i>M. orthorhizidis</i> <i>M. cloacale</i> , <i>M. falconis</i> <i>M. faucium</i> , <i>M. hominis</i> <i>M. hyosynoviae</i> , <i>M. orale</i> <i>M. salivarium</i>	MP-CA1 MP-CA2	MWTVG TRTCCAG TTTTGAGAG TTTAGATCAACCTATAGAATA	9 10
<i>M. bovis</i> , <i>M. fermentans</i> <i>M. opalescens</i> , <i>M. primatum</i> , <i>M. spermatophilum</i> , <i>M. synoviae</i>	MP-CB1 MP-CB2 MP-CB3 MP-CB4	RTATYTAGTTTGAGAGFRCA WWTRATTYATTTAAATGTCTT GGKYAA TTG TTTWGT RTATTTTACAMCGMCAYC	11 12 13 14
<i>M. muris</i> , <i>M. penetrans</i> <i>U. urealyticum</i>	MP-CC1 MP-CC2	CC TCCTTTC TATCGGAGTAMA CGGATTC TATTTAG TTTTGAG	15 16
<i>M. neurolyticum</i> , <i>M. pulmonis</i>	MP-CD1 MP-CD2 MP-CD3	TAAAATAGATACCTTAAKATA GTATYYAGTTTGAAG CTTGCCAAWTAGWTWT	17 18 19
<i>M. genitalium</i> , <i>M. plium</i> <i>M. pneumoniae</i>	MP-CE1 MP-CE2	AWACRACAA TCTTTC TAGTTC AATAAGTTAC TAAGGC TAT	20 21
<i>Acholeplasma</i>	AP-CP1 AP-CA1 AP-CA2 AP-CA3 AP-CB1 AP-CB2	TCATCATATTCAG TTTTG GGGCC TRTAGCTCAGYTG GTT AGAGCFCWC GCYTGATAAGCG WFRGG TCGATGG TTTTCAG TCC TCATCATATTCAG TTTTGARR AGTC TTTGAAAAGTAGATAAA	22 23 24 25 26 27

【Table 3】

Species	Probe	Sequence	SEQ ID NO.
<i>M. arginini</i>	MP-arg1	AGATTATATCATACAATAGA	28
	MP-arg2	GAGTACATAAAATGTTATGGAA	29
<i>M. arthritidis-faucium</i>	MP-arf1	TGAAGCCCGATGGTGCTTGG	30
	MP-arf2	TGAGAGAAC TAAACTTC TCTC	31
	MP-arf3	GAATACAAAA TCAATACAATA	32
<i>M. fermentans</i>	MP-fer1	ATG TAC TATTAAC TATTTCAC	33
	MP-fer2	TACAAAAGAG TAC TTTTAA	34
	MP-fer3	TTTTTATGGGTC TAAAGCTTT	35
	MP-fer4	GAACAAATATTTT TTTCTC TCA	36
	MP-fer5	ATAACAAACTATAACAATAGG	37
<i>M. hominis</i>	MP-hom1	ATTTATCTC TCGGTCTTT	38
	MP-hom2	ATATTTATATTTTATAAGACA	39
	MP-hom3	ATTGATATATTAATTAATATT	40
<i>M. hyorhinis</i>	MP-hyo1	G AATAGCAAATAACAATATGATT	41
	MP-hyo2	CGGAGTACATTAGTCTTAATT	42
	MP-hyo3	TTACATAATCGATTCGTGCTCT	43
	MP-hyo4	AGCTTTAAGTCTCTAATTATA	44
	MP-hyo5	TTCATATTTATTTTCAACG	45
	MP-hyo6	AACGATCTTTTATAACCGA	46
	MP-hyo7	TTAAATTCTAAAAATAGATTA	47
	MP-hyo8	AGATATTTATCTTTAGCAATA	48
<i>M. neurolyticum</i>	MP-neu1	GG TTATTATGGGCTTGCTA	49
	MP-neu2	GGTTATTTAAAAATCC TTTTA	50
	MP-neu3	TAAATTTTCTTCTCAATTAA	51
<i>M. opalescens</i>	MP-opa1	CATCATAATGTAACCAATAC	52
	MP-opa2	ACAAAAATCATTA TTTTAAAT	53
	MP-opa3	TTTAATGATTATTAACTTTT	54
	MP-opa4	TTATGTGCTTTG TTTTATG G	55
	MP-opa5	TATGGTCTACAAAGCTTATAT	56
	MP-opa6	GATAAAAAATCATATAAATT	57
<i>M. orale</i>	MP-ora1	CATAAATAG TTAATGGCTCA	58
	MP-ora2	ATAGAGACAAAATACAAAAACA	59
	MP-ora3	GG TCAAAAATACTTATACGTA	60
<i>M. pirum</i>	MP-pir1	TAGTCTTTTG TG TGAATAACA	61
	MP-pir2	CTTTATACACC TTATTACAAT	62
	MP-pir3	TAAAAATCCAAATTAAATGTTA	63
	MP-pir4	GCAAATTTGATG TCAACATTT	64
	MP-pir5	AATTAATCTCTCCTATTACTT	65

	MP-pir6	TTAAAG TAGTAGAGATGG TTC	66
	MP-pir7	CAAATATCAAAATGC TAA TGGA	67
	MP-pir8	ATGC TAA TGG ATA TCAAAAAA	68
<i>M. penetrans</i>	MP-pen1	AAGAGTAAGTTC TAG GTCG	69
	MP-pen2	CATTAAAGCTAAG TAACAAAT	70
	MP-pen3	TCC TAAAC TGAATTTTATCT	71
	MP-pen4	TATATAAAGATAGGTTC TAG	72
	MP-pen5	ATTTTTC TC TCAAGATAGTTC	73
	MP-pen6	TC TAATCATATTCG TTATTTT	74
<i>M. pulmonis</i>	MP-pul1	AATTTTGTATCCGAGTCATT	75
	MP-pul2	CATTTTCTATCAATAGTTAT	76
	MP-pul3	TATG TGTATC TTGCCAATTAG	77
	MP-pul4	TCTATCTTTCAAAACAAATA	78
	MP-pul5	TATAAATTAATATGATAACGT	79
	MP-pul6	TCATCAAAATGTAAAAATTTT	80
	MP-pul7	AAAAA TAAAA TAGATACCTTA	81
	MP-pul8	AAATAAA TTCAACAA TAGGA	82
<i>M. salivarium</i>	MP-sal1	TAATGGATT TAA TTTTCGTG	83
	MP-sal2	TATCAAAATCAATATAATATT	84
<i>M. cloacale</i>	MP-cla1	AGTACAATTCTCAC TGTATAG	85
	MP-cla2	TAGAATATCAAGACATATAC	86
<i>M. falconis</i>	MP-fal1	GAGTACAAC TTCTGTATAG	87
	MP-fal2	AGAA TACAAAAA TAGACAA	88
	MP-fal3	AT TGA AAAATTA TTAATTAAT	89
<i>M. hyosynovise</i>	MP-hyos1	CTAGACTAAAG TTAATGGTAC	90
	MP-hyos2	AA TTA TCAAAATTA TTTTCA	91
<i>M. muris</i>	MP-mur1	TATAGAAAAACCCACATCA	92
	MP-mur2	TATAGAAATATTTTAAATATT	93
	MP-mur3	GATTATTACACCATATAGAA	94
	MP-mur4	TCAATAAACCTAAATAAAAAA	95
<i>M. primatum</i>	MP-pri1	GTAGACATAACCCAGC TA	96
	MP-pri2	CAAACGCTATCGCTTTTATG	97
	MP-pri3	TCATGGGCTTTTAA TAGGGTC	98
	MP-pri4	ACCCCAAC TCCCATCAAAAT	99
<i>M. spermophilum</i>	MP-spe1	TTCATCGAGATAGTCATTTTA	100
	MP-spe2	CAAAATATCATTCAAATTTT	101
	MP-spe3	TTTGTAC TGAA TGTATTAAC	102
	MP-spe4	TTGTTATG TGAC TTTTATGG	103
	MP-spe5	AAAACAAACAACTATACAAT	104

<i>M. synoviae</i>	MP-syn1	TTGGCTTGGGCTATTGTATT	105
	MP-syn2	GCGGTGTGTATCGCTTTTT	106
	MP-syn3	ACCTCTCTTAAAAATTGTTCT	107
	MP-syn4	CCGAGTTTAAATTATTGAAAT	108
	MP-syn5	CATCA TAACAACA TAACAATA	109
<i>M. pneumoniae</i>	MP-pne1	GTAAATTAAACCCAAATCCC	110
	MP-pne2	ATCTTTAATAAAGATAAATAC	111
	MP-pne3	CTAAACAAAAACA TCAAAATCC	112
	MP-pne4	AAAGAACA TTTCCGC TCTTT	113
<i>M. genitalium</i>	MP-gen1	CACCCCTTAATTTTTTCGG	114
	MP-gen2	AATGGAGTTTTTATTTTTATTTA	115
	MP-gen3	CCCAAATCAAATG TTTGG TCTC	116
	MP-gen4	CAACTAACACAC TGG TCAGT	117
	MP-gen5	AGAAATGTTTTGAACAGTTC	118
	MP-gen6	TAGTTCCAAAAA TAAATACCA	119
<i>M. bovis</i>	MP-bov1	TATAACCAAAATTAAGACCTTA	120
	MP-bov2	GTCATGGCTTTTATTAATAGG	121
<i>U. urealyticum</i>	UP-ure1	CATTAAAGTTGTCAGTGAA	122
	UP-ure2	TAATTTACG TACTAATAAGTG	123
	UP-ure3	TTTATTAATAATCCATA TGAAT	124
	UP-ure4	AAGCCAC TTTT TAAAAATTT	125
	UP-ure5	CCATAATAATTAATTTATAT	126
	UP-ure6	ATTATCAACAAATCTTTC TAA	127
<i>A. laidlawii</i>	AP-lai1	AACAC TTAGCACAGATGAC	128
	AP-lai2	CTTTC TAAGGAGAAAGGC TAA	129
	AP-lai3	ATGAC TAC TAG TAAG TAG TAA	130
	AP-lai4	GTAG TAA TATTC TC TAAATT	131
	AP-lai5	TAAAGTAA TTTAAGTGTTTC	132
	AP-lai6	TAAATGATGTCTGAAAAGAAA	133

* Mixed Base $\frac{1}{2}$ Code Name

M : A + C, W : A + T, Y : C + T, R : A + G
K : G + T, V : G + A + C, N : A + G + C + T

5

Brief Description of the Drawings

FIGS. 1a to 1f show multiple sequence alignments of each ITS region of *Mycoplasma* and *Ureaplasma* for selecting genus-specific probes.

FIGS. 2a to 2c show multiple sequence alignments of each ITS region of *Acholeplasma* for selecting genus-specific probes.

FIG. 3 shows a result of PCR amplification using primer pairs which can amplify ITS target sequences of many *Mycoplasma* and its related strains

FIG. 4 shows a microarray comprising probes for detecting genotypes of *Mycoplasma* and its related strains as a set on a support.

FIGS. 5a to 5k show results of image analysis of specific hybridization reaction of each probes for detecting genotypes of *Mycoplasma* and its related strains and results of numerical analysis calculated from pixel intensity.

5

Best mode for carrying out the Invention

The present invention will be described in greater detail by means of following examples. The following examples are for illustrative purpose and are not intended to limit the scope of the invention.

10

Example 1: Incubation of *Mycoplasma* and its related strains and Isolation of Genomic DNA

Total 25 kinds of strains, including 1 kind of *Acholeplasma*, 23 kinds of *Mycoplasma*, and 1 kind of *Ureaplasma* were obtained from the American Type Culture Collection (ATCC). The strains were cultured in each culturing media under each culturing conditions according to manual provided by ATCC. From the cultured media, strain colonies were obtained with a white gold ear and input in 1.5ml tube, 100 μ l of InstaGene matrix (Bio-Rad, USA) was added thereto and suspended, and reaction was performed at 56°C for 30 minutes in constant temperature bath. And then, the reactant was shook for 10 seconds, heated at 100°C for 8 min, shook again for 10 sec, centrifuged at 12,000 rpm for 3 min, transferred to new tube, and freeze-stored at -20°C. The product was used as template DNA of PCR reaction.

25

The strains used were as followed:

Acholeplasma laidlawii (ATCC 25937)

Mycoplasma arginini (ATCC 23838)

Mycoplasma arthritidis (ATCC 19611)

Mycoplasma bovis (ATCC 27368)

30

Mycoplasma cloacale (ATCC 35276)

- 5 *Mycoplasma falconis* (ATCC 51372)
 Mycoplasma faucium (ATCC 25293)
 Mycoplasma fermentans (ATCC 19989)
 Mycoplasma genitalium (ATCC 33530)
 Mycoplasma hominis (ATCC 23114)
 Mycoplasma hyorhinis (ATCC 17981)
 Mycoplasma hyosynoviae (ATCC 25591)
 Mycoplasma muris (ATCC 33757)
10 *Mycoplasma neurolyticum* (ATCC 19988)
 Mycoplasma opalescens (ATCC 27921)
 Mycoplasma orale (ATCC 23714)
 Mycoplasma penetrans (ATCC 55252)
 Mycoplasma pirum (ATCC 25960)
 Mycoplasma pneumoniae (ATCC 15531)
15 *Mycoplasma primate* (ATCC 15497)
 Mycoplasma pulmonis (ATCC 14267)
 Mycoplasma salivarium (ATCC 23064)
 Mycoplasma spermatophilum (ATCC 49695)
 Mycoplasma synoviae (ATCC 25204)
20 *Ureaplasma urealyticum* (ATCC 27618)

Example 2: Preparation of probes for detection of *Mycoplasma* and its related strains

- 25 The probes used for detection of *Mycoplasma* and its related strains were selected based on a result of multiple alignment of ITS sequences of *Mycoplasma*, *Acholeplasma* and *Ureaplasma*. Among *Mycoplasma* and its related species, 16S rRNA sequences has high similarity of 74~97%, whereas ITS sequences has lower similarity of 25.4~78.8% except for between *M. salivarium* and *M. hyosynoviae*, and
30 *M. hominis* and *M. falconis*. In other words, ITS contains a region more polymorphic than 16S rRNA which is useful for designing probes for

detection of *Mycoplasma* and its related strains. However, to complement specificity between *M. salivarium* and *M. hyosynoviae*, and *M. hominis* and *M. falconis* having a high ITS similarity, more restrictive and strict probes were designed.

5 In the present invention, the oligonucleotide probes for detection of *Mycoplasma* and its related strains were prepared by synthesizing 15-25 bases of specific probe with 15 bases of dT spacer at 5' end. Probes for detection of *Mycoplasma* and its related strains are not restricted to the sequences disclosed in Tables 2 and 3 and any primer
10 and probes comprising the sequences can be used in the present invention.

1. Preparation of probes for detection of *Mycoplasma* and *Ureaplasma*

15 ① Preparation of probes for genus-specific detection of *Mycoplasma* and *Ureaplasma*

For genus-specific hybridization with all *Mycoplasma* and *Ureaplasma* genus, probes of SEQ ID Nos. 7 and 8 in Table 2 were designed from conserved sequences of ITS of *Mycoplasma*. Further,
20 each Group-based conserved sequences targeted to *Mycoplasma* ITS were designed as follows. For detecting Group I (*M. arginins*, *M. arthritidis*, *M. cloacale*, *M. falconis*, *M. faucium*, *M. hominis*, *M. hyosynoviae*, *M. orale*, *M. salivarium*), probes of SEQ ID Nos. 9 and 10 were designed. For detecting Group II (*M. bovis*, *M. fermentans*, *M.*
25 *opalescens*, *M. primatum*, *M. spermatophilum*, *M. synoviae*), probes of SEQ ID Nos. 11, 12, 13 and 14 were designed. For detecting Group III (*M. muris*, *M. penetrans*, *U. urealyticum*), probes of SEQ ID Nos. 15 and 16 were designed. For detecting Group IV (*M. neurolyticum*, *M. pulmonis*), probes of SEQ ID Nos. 17, 18 and 19 were designed. For
30 detecting Group V (*M. genitalium*, *M. pirum*, *M. pneumoniae*), probes of

SEQ ID Nos. 20 and 21 were designed.

② Preparation of probes for species-specific detection of
Mycoplasma and *Ureaplasma*

For species-specific hybridization with each *Mycoplasma* and
Ureaplasma species, 100 kind of probes of SEQ ID Nos. 28 to 127 in
Table 3 were designed from species-specific sequences of ITS of
Mycoplasma and *Ureaplasma*, which can detect 25 kind of *Mycoplasma*
strains.

2. Preparation of probes for detection *Acholeplasma*

① Preparation of genus-specific probes for detection
Acholeplasma

For genus-specific hybridization with all *Acholeplasma* genus,
probes of SEQ ID No. 22 in Table 2 was designed from conserved
sequences targeted to both of ITS1 and ITS2 of *Acholeplasma*. Further,
each Group-based conserved sequences targeted to each
Acholeplasma ITS1 and ITS2 were designed as follows. For Group I
targeted to ITS1, probes of SEQ ID Nos. 23, 24 and 25 were designed.
For Group II targeted to ITS2, probes of SEQ ID Nos. 26 and 27 were
designed.

② Preparation of species-specific probes for detection
Acholeplasma

For species-specific hybridization with each *Acholeplasma*
species, probes of SEQ ID Nos. 128 to 133 in Table 3 were designed
from species-specific sequences of ITS of *Acholeplasma*.

Example 3: Preparation of target DNA

1. Preparation of target DNA for detection of *Mycoplasma* and its

related strains

For preparing target DNA for detection of *Mycoplasma* and its related strains, 187~290bp size of ITS regions were selectively amplified using 5'-biotin-GTG(C/G)GG(A/C)TGGATCACCTCCT-3' (MP16SF-2) and 5'-biotin-GCATCCACCA(A/T)A(A/T)AC(C/T)CTT-3' (MP23SR-2), and 5'-biotin-AAAGTGGGCAATACCCAACGC-3' (M78) and 5'-biotin-CCACTGTGTGCCCTTTGTTTCCT-3' (R34) which were biotin-labeled respectively (Tang et al., 2000.). To prepare genomic DNAs of *Mycoplasma* and its related strains isolated in Example 1, PCR were carried out using the above primers in the following conditions: denaturation at 94°C for 3 minutes, 30 cycles of amplification at 94°C for 30 seconds, at 55°C for 2 minutes and at 72°C for 2 minutes, and final extension at 72°C for 10 minutes. After the reaction, the reaction products were analyzed by ELECTROPHORESIS on a 2% agarose gel. FIG. 3 is an electrophoresis image taken after the PCR performed using primers capable to amplify ITS target sequences of several *Mycoplasma*.

Example 4: Probe immobilization on support

Among the probes prepared in Example 2, each representative probes for *Mycoplasma*, *Acholeplasma* and *Ureaplasma* were selected. Each of the selected probes was transferred to 384-well microplate, diluted to a concentration of 50 pmole by adding spotting solution, and immobilized on a slide glass using a microarrayer (Cartesian Technologies, USA). In FIG 4, each probes for detection of *Mycoplasma* and its related strains correspond to SEQ ID Nos. 7, 28, 30, 33, 38, 41, 49, 52, 58, 61, 69, 75, 83, 85, 87, 30, 90, 92, 96, 100, 105, 110, 114, 120, 122, 22, 128, and 7 in order. Two spots of each kind of the probes were attached to the support and left in a slide box at room temperature for 24 hours or in a dry oven at 50°C for about 5 hours to be fixed to the surface of the support.

Example 5: Unimmobilized probe washing

The slide glass after the process in Example 4 was washed with a 0.2% SDS buffer solution and then distilled water at room temperature to remove unimmobilized probes. The washed slide glass was immersed in a sodium borohydride (NaBH_4) solution for 5 minutes and then washed again at 100°C . Final washing with a 0.2% SDS solution and then distilled water was followed by centrifugation to fully dry the slide glass.

Example 6: Hybridization

The biotin-labeled target products prepared in Example 3 were thermally treated to be denaturated into single strands and cooled to 4°C . A hybridization reaction solution containing $2\mu\text{l}$ of the target products was prepared. This hybridization reaction solution was portioned on the slide glass after the process in Examples 4 and 5, and the slide glass was covered with a cover slip and reacted at 25°C for 1 hours.

Example 7: Unhybridized target DNA washing

TO WASH OUT UNHYBRIDIZED TARGET DNAs, THE COVER SLIP WAS REMOVED USING A 2X SSC WASHING SOLUTION (300MM NaCl, 30MM Na-CITRATE, PH 7.0), AND THE SLIDE WAS WASHED WITH 2X SSC AND THEN 0.2X SSC, FOLLOWED BY CENTRIFUGATION TO FULLY DRY THE SLIDE GLASS.

Example 8: Staining and Result analysis

To determine hybridization of PCR products and probes, Cy5-streptavidin or Cy3-streptavidin (Amersham pharmacia biotech, USA) was diluted with 6x SSC and BSA (Bovine Serum Albumin), about $40\mu\text{l}$ of dilutes was portioned on slide glass, and the slide glass was covered

with a cover slip to block light and reacted at 50°C for about 20 minutes. After the reaction, the cover slip was removed using a 2X SSC solution, and the slide was washed with 2X SSC and then 0.2X SSC. The hybridized result was scanned using a non-confocal laser scanner (GenePix 4000A, Axon Instruments, U.S.A.) and analyzed by image analysis.

FIG. 5 shows results of image analysis of specific hybridization reaction of each probes for detecting genotypes of representative 11 kinds of *Mycoplasma* and its related strains and results of numerical analysis calculated from pixel intensity.

FIG. 5a shows results of hybridization reaction of genus-specific probe (SEQ ID No. 7) and species-specific probe (SEQ ID No. 85) of *M. cloacale*. FIG. 5b shows results of hybridization reaction of genus-specific probe (SEQ ID No. 7) and species-specific probe (SEQ ID No. 87) of *M. falconis*. FIG. 5c shows results of hybridization reaction of genus-specific probe (SEQ ID No. 7) and species-specific probe (SEQ ID No. 90) of *M. hyosynoviae*. FIG. 5d shows results of hybridization reaction of genus-specific probe (SEQ ID No. 7) and species-specific probe (SEQ ID No. 49) of *M. neurolyticum*. FIG. 5e shows results of hybridization reaction of genus-specific probe (SEQ ID No. 7) and species-specific probe (SEQ ID No. 52) of *M. opalescens*. FIG. 5f shows results of hybridization reaction of genus-specific probe (SEQ ID No. 7) and species-specific probe (SEQ ID No. 69) of *M. penetrans*. FIG. 5g shows results of hybridization reaction of genus-specific probe (SEQ ID No. 7) and species-specific probe (SEQ ID No. 61) of *M. pirum*. FIG. 5h shows results of hybridization reaction of genus-specific probe (SEQ ID No. 7) and species-specific probe (SEQ ID No. 83) of *M. salivarium*. FIG. 5i shows results of hybridization reaction of genus-specific probe (SEQ ID No. 7) and species-specific probe (SEQ ID No.

100) of *M. spermatophilum*. FIG. 5j shows results of hybridization reaction of genus-specific probe (SEQ ID No. 7) and species-specific probe (SEQ ID No. 122) of *U. urealyticum*. FIG. 5k shows results of hybridization reaction of genus-specific probe (SEQ ID No. 22) and species-specific probe (SEQ ID No. 128) of *A. laidlawii*.

Industrial Applicability

As described above, the present invention provides a rapid and accurate assay method capable of simultaneously detecting many *Mycoplasma* and its related strains from a single sample using a microarray comprising novel oligonucleotides for detecting *Mycoplasma* and its related strains which are known as a source of contamination of cell lines and biological products and human pathogenic.

Also, the present invention provides an objective and credible assay method capable of tracing a contamination source for preventing expansion of infective *Mycoplasma* and its related strains and controlling a contamination of *Mycoplasma* against biological products and stem cells or cord blood cells which are useful for gene therapy and cell therapy.

Further, the present invention provides very specific and sensitive hybridization assay for detecting *Mycoplasma* and its related strains using oligonucleotide probes designed based on sequence analysis of ITS region of many *Mycoplasma* Strains.

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What is claimed is:

1. An ITS (Internal transcribed spacer) target DNA for genotyping *Mycoplasma* strains, comprising any one sequence selected from SEQ ID Nos. 1 to 6.
5
2. An oligonucleotide for genus-specific genotyping of *Mycoplasma* and *Ureaplasma* strains, comprising any one sequence selected from SEQ ID Nos. 7 to 21 or its complementary sequence.
10
3. An oligonucleotide for species-specific genotyping of *Mycoplasma* and *Ureaplasma* strains, comprising any one sequence selected from SEQ ID Nos. 28 to 127 or its complementary sequence.
- 15 4. An oligonucleotide for genus-specific genotyping of *Acholeplasma* strains, comprising any one sequence selected from SEQ ID Nos. 22 to 27 or its complementary sequence.
- 20 5. An oligonucleotide for species-specific genotyping of *Acholeplasma* strains, comprising any one sequence selected from SEQ ID Nos. 128 to 133 or its complementary sequence.
- 25 6. A microarray comprising more than one oligonucleotide selected from genus-specific and species-specific oligonucleotides for genotyping *Mycoplasma*, *Acholeplasma* and *Ureaplasma* strains according to any one from claims 2 to 5 as probes attached on a support.
7. The microarray according to claim 6, wherein the probes are any one selected from a group consisting of DNA, RNA, PNA, LNA and HNA.
30
8. The microarray according to claim 6, wherein the support is any

one selected from a group consisting of slide glass, plastic, membrane, semiconductive chip, silicon and gel.

5 9. A method for detecting *Mycoplasma* strains, comprising the following steps:

a) extracting nucleic acids from a sample;

b) amplifying target DNA among the extracted nucleic acids;

c) hybridizing the amplified target DNA with probes of the microarray according to claim 6; and

10 d) detecting signals generated from the hybridization reaction.

10. A kit for diagnosing *Mycoplasma* infection, comprising more than one oligonucleotide selected from genus-specific and species-specific oligonucleotides for genotyping *Acholeplasma*, *Mycoplasma* and
15 *Ureaplasma* strains according to any one from claims 2 to 5.

Abstract of the Invention

The present invention relates to a method for detecting *Mycoplasma* and its related strains which are source of contamination of cell lines and biological products and human pathogenic. More particularly, the present invention relates to genus-specific and species-specific oligonucleotides for genotyping of *Mycoplasma*, *Acholeplasm* and *Ureaplasma* strains, microarray comprising the oligonucleotides, and method for detection of species using the microarray.

As described above, the present invention provides a rapid and accurate assay method capable of simultaneously detecting many *Mycoplasma* and its related strains from a single sample using a microarray comprising novel oligonucleotides for detecting *Mycoplasma* and its related strains which are known as a source of contamination of cell lines and biological products and human pathogenic. Further, the present invention provides an objective and credible assay method capable of tracing a contamination source for preventing expansion of infective *Mycoplasma* and its related strains and controlling a contamination of *Mycoplasma* against biological products and stem cells or cord blood cells which are useful for gene therapy and cell therapy.

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FIG. 1a

<i>M. bovis</i>	---ATA-----	TGTTCTTTGAAAACTGAATAGTAAAAATTTTT	142
<i>M. primetus</i>	---TT-----	TGTTCTTTTGAAAACTGAATAGTAAAAATTTTT	181
<i>M. forasense</i>	---ATT-----	TGTTCTTTGAAAACTGAATAGTAAAA---TTTTT	177
<i>M. upilevense</i>	---TT-----	TGTTCTTTGAAAACTGAATAGTAAAA---TTTTA	159
<i>M. apertrophium</i>	---AT-----	TGTTCTTTGAAAACTGAATAGTAAAA---TTTTT	196
<i>M. synoviae</i>	---AAI-----	TGTTCTTTGAAAACTGAATAGTAAAA---IAA	100
<i>M. neurolyticum</i>	TAATAAATGTTTT---AAT	TGTTCTTTGAAAACTGAATAGTAAAA---TA---T	176
<i>M. pulchra</i>	---AACAAATA-----	GTTCCTTTGAAAACTGAATAGTAAAA---TAAAT	159
<i>M. hyorhinis</i>	---ATA-----	GTTCCTTTGAAAACTGAATAGTAAAA---TAA	112
<i>M. orthorhinis</i>	---TT-----	TGTTCTTTGAAAACTGAATAGTAAAA---T	115
<i>M. fauveli</i>	---TT-----	TGTTCTTTGAAAACTGAATAGTAAAA---T	123
<i>M. orele</i>	---II-----	TGTTCTTTGAAAACTGAATAGTAAAA---I	108
<i>M. hyosynoviae</i>	---TT-----	TGTTCTTTGAAAACTGAATAGTAAAA---T	119
<i>M. salivarium</i>	---TT-----	TGTTCTTTGAAAACTGAATAGTAAAA---T	115
<i>M. falconis</i>	---TT-----	TGTTCTTTGAAAACTGAATAGTAAAA---T	92
<i>M. luscini</i>	---TT-----	GTTCCTTTGAAAACTGAATAGTAAAA---T	07
<i>M. arginini</i>	---TT-----	TGTTCTTTGAAAACTGAATAGTAAAA---T	93
<i>M. oloresale</i>	---II-----	TGTTCTTTGAAAACTGAATAGTAAAA---I	96
<i>M. garrulatus</i>	CCAGTTCTGAAAG---AATGTTTTGAA	AGTTCTTTGAAAACTGAATAGTAAAA---GACA	160
<i>M. pseudotuberculosis</i>	CCAGTTCTGAAAG---AATGTTTTGAA	AGTTCTTTGAAAACTGAATAGTAAAA---GACA	190
<i>M. pirus</i>	TAAATTTTAAAGTAGTAGATGG	TTCTTTGAAAACTGAATAGTAAAA---GACA	213
<i>M. musis</i>	TT-----	CTTTGAAAACTGAATAGTAAAA---GACA	106
<i>M. penetans</i>	TT-----	CTTTGAAAACTGAATAGTAAAA---GACA	184
<i>U. urealyticum</i>	IIAATIIAIIATG---GATGATGGA	CTTTGAAAACTGAATAGTAAAA---GACA	199

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FIG. 1b

<i>M. arthritidis</i>	AAA—CATCGTATCCAGTTTTSAGAGAACTAACTCTCTCTTTGTTCTTTGAAAC	108
<i>M. faucius</i>	AAA—CATCGTATCCAGTTTTSAGAGAACTAACTCTCTCTTTGTTCTTTGAAAC	88
<i>M. falconis</i>	TAA—ATTTGTATCCAGTTTTSAGAGAACTA—ACTCTCTTTT—GTTCTTTGAAAC	85
<i>M. hominis</i>	AAAAAA—ATTTGTATCCAGTTTTSAGAGAACTA—TCCTCG—GTTCTTTGAAAC	80
<i>M. arginini</i>	AAA—T—ATTTGTATCCAGTTTTSAGAGAACTA—TCCTCAATTT—GTTCTTTGAAAC	86
<i>M. cloacae</i>	GAATTA—ATTTGTATCCAGTTTTSAGAGAACTT—TCCTCAATTTGTTCTTTGAAAC	89
<i>M. hyosynoviae</i>	CA—ATTCGTATCCAGTTTTSAGAGAACTAT—TCCTCTTTT—GTTCTTTGAAAC	113
<i>M. oris</i>	CAA—ATTCGTATCCAGTTTTSAGAGAACTAT—CTCTCATTT—ATTCTTTGAAAC	102
* * ***** *		
<i>M. arthritidis</i>	—TTAAAAAATTAATATTTCAA—GTTTAGATCAAOCATAGAATACAA	173
<i>M. faucius</i>	—TTAAAAAATTAATATTTCAA—GTTTAGATCAAOCATAGAATACAA	153
<i>M. falconis</i>	ATTA—TTAATTAATATTTCAA—GTTTAGATCAAOCATAGAATACAA	150
<i>M. hominis</i>	—TA—TTAATTAATATTTCAA—GTTTAGATCAAOC—ATAGAATATTT	141
<i>M. arginini</i>	ATTAATTTTATTAATATTTCAA—GTTTAGATCAAOCATAGAATATAT	153
<i>M. cloacae</i>	—TCAATAAATTAATATTTCAAATGTTTAGATCAAOCATAGAATATTC	154
<i>M. hyosynoviae</i>	A—TTATCAAATTAATATTTCAA—GTTTAGATCAAOCATAGAATATTC	178
<i>M. oris</i>	—TTAAAAATTAATATTTCAAAA—ATTTAGATCAAOCATAGAATATTC	168
***** * ***** * *****		

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FIG. 1c

<i>M. bovis</i>	TTATT AATAGBCTAAAGCTA	ATATCTAGT TTTGAGGAGCA	—TCTCTCAT	144
<i>M. primatum</i>	TT—TAATAGBCTGAGCTT	ATATCTAGT TTTGAGGAGCA	—TCTCTCTT	148
<i>M. fermentans</i>	TTTTTATGAGBCTAAAGCTT	ATATCTAGT TTTGAGGAGCA	ATATTTTCTCTCAT	148
<i>M. opalescens</i>	T—ATBGTCTACAAGCT	ATATCTAGT TTTGAGGAGCA	—TCTCTCTT	129
<i>M. spermatophilum</i>	TT—TTATGBCCTAAAAGTCT	ATATCTAGT TTTGAGGAGCA	—TCTCTCTAAT	166
<i>M. synoviae</i>	GCTTT TTTTGCCTTGGCTAT	GTATTTAGT TTTGAGGAGCAC	—CTCTCTTAAA	141
	* *	* ** *	*****	**
<i>M. bovis</i>	ATGTTCTTTGAAAAGCTGAATAGTAAATATTTTTCATATTTACAGGACATGAAA—	201		
<i>M. primatum</i>	—TGTTCCTTTGAAAAGCTGAATAGTAAATATTTTTCATATTTACAGGACATGAAA—	207		
<i>M. fermentans</i>	TTGTTCTTTGAAAAGCTGAATAGTAAATTTTTCATATTTACAGGACATGAAA—	200		
<i>M. opalescens</i>	—TGTTCCTTTGAAAAGCTGAATAGTAAATTTTTCATATTTACAGGACATGATA—	182		
<i>M. spermatophilum</i>	—TGTTCCTTTGAAAAGCTGAATAGTAAATTTTTCATATTTACAGGACATGAAA—	219		
<i>M. synoviae</i>	TTGTTCTTTGAAAAGCTAAATAGTAA—TAAATATTTACAGGACATGAAAAT—	199		
	*****	*****	*	*****
<i>M. bovis</i>	—ATCAAA—TTAATGGTAATTTGTTTTGAGT—CATCGAGT—AAGTCATATTTA—	250		
<i>M. primatum</i>	CCATCAAAAAATTAAATGGTAATTTGTTTTGAGT—CATCGAGT—AAATCATATTTAA—	261		
<i>M. fermentans</i>	—TTAAA—TTAATGGTAATTTGTTTTGAGT—CATCGAGT—AAATCATATTTAA—	250		
<i>M. opalescens</i>	—ATTAAATGATTTA—GGTAATTTGTTTTGAGT—CATCGAGT—AAATCAATCATATAA—	236		
<i>M. spermatophilum</i>	TAATTGAA—TTAA—GGTAATTTGTTTTGAGT—CATCGAGT—TAGTCATTTTAAA—	270		
<i>M. synoviae</i>	—ATAAATTAATTAA—GGTAATTTGTTTTGAGT—ACCGAGTT—TAAATTAT—TGAA—	243		
	* * *	* *	*****	***
<i>M. bovis</i>	—TATGATTCATGAAATGCTT—AAATACACATCTAA—	304		
<i>M. primatum</i>	—TATGATTCATGAAATGCTT—AAATACACATCTTAA—	319		
<i>M. fermentans</i>	—TATGATTCATGAAATGCTT—AAATACACATCTAA—	306		
<i>M. opalescens</i>	TTTTCATTCATGAAATGCTT—AAATACACATCTAA—	296		
<i>M. spermatophilum</i>	AAATGATTCATGAAATGCTT—AAATACACATCTAA—	330		
<i>M. synoviae</i>	—AAATGATTCATGAAATGCTT—AAATACACATCTAA—	295		
	* ** *	*****	**	*****

FIG. 1d

<i>M. muris</i>	CCCTCCTTTCTATCGAGTACA	TTTAGATTATTACACCATATTAGAAATATTTTAAATATT	80
<i>M. penetrans</i>	CCCTCCTTTCTATCGAGTACA	TAAAGCTAAGTAACAAATATTAG-----ATATATT	82
<i>U. urealyticum</i>	CCCTCCTTTCT-TGGGTAAA	TTTTAAT--TTACGTACTAATAAG--TGACATTTT	83
	*****	*****	

<i>M. muris</i>	TGTGTACTTT-TTATAGAAAACCCCAATCAATAAAGCTAA-----ATAAAAAATTATTT	115
<i>M. penetrans</i>	TGTGTACTTTATTAATAAAATCCTAAACTGAAATTATCTCATGTTATATAAGAGTAAGT	112
<i>U. urealyticum</i>	TATTAAATCCATATGAATATAAGCCACTTTTTTAAAAATTTT-----TAAAAATTCATAT	109
	* * * * *	

<i>M. muris</i>	TTTGGC-CGGATTCTATTTAGTTTGAAG	GATA-TTTTCTCTCATGATAGTT-----	165
<i>M. penetrans</i>	TCTAGC-CGGATTCTATTTAGTTTGAAG	GAT-TTTTCTCTCAAGATAGTT-----	162
<i>U. urealyticum</i>	---GC-CGGATTCTATTTAGTTTGAAG	ATTATTCTCTCCCAATAATAATTAAATTATT	165
	** *****		

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FIG. 1e

M. pulmonis CTACGGAGTACAAAACCATTTTTTTAGAAATGGCATTTT—TCTATCAATAGTTAT— 54
M. neurolyticum CTACGGAGTACACATACATCTTATTAATTTGGTTATTTAAAAATCCCTTTTATAATAAAT 60
 ***** * *** * * * * * * * * * *

M. pulmonis —AGAAAGTCCTTATGTGTCTCTGCCAATTAGATATCTAGTATTCACTTTTGAAAGTCT 113
M. neurolyticum AAAAAAGGTTATTATGGCCTTGCCAAATAG—TTTGTATCTAGTTTGTGAAGTTT 114
 * * * * * * * * * * * * * * * * * * *

M. pulmonis A—TCTTTCAAA—ACAAATA—GTCTTTAAAACTGAATAGCATAT 155
M. neurolyticum AATTTTTTCTTTCTAATTAATAAATGTTTTAATATATTCCTTTGAAAACCTGAATAGCAAAAT 174
 * * * * * * * * * * * * * * * * * *

M. pulmonis AAATTAATATGATAACGTCATCAAAATGTAAATTTTTGATCCGAGTCATTTTAAACAA 215
M. neurolyticum —ATTGAAATTTTAATTTTCATAAATTTCAACAACGACATTACAACACCGAGTCTAACTG 232
 * * * * * * * * * * * * * * * * * *

M. pulmonis TTT—GTAAAAAAT—AAAATAGTACCTTAAG—ATAACATCAAAAA—ATAAAT 265
M. neurolyticum TTTTATTGAACAGTTAGCTTAAATAGTACCTTAGATATAAATCTAAACAATAGGC 292
 *** * * * * * * * * * * * * * * * * *

FIG. 1f

M. pneumoniae AACATTTCCGC—TTCTTTCAAAACTGAAACGACAA—TCTTCTAGTTCCA— 205
M. genitalium AATGTTTTGAAACAGTTCTTTCAAAACTGAAACGACAA—TCTTCTAGTTCCA— 175
M. pirum AGTAGAGATGG—TTCTTTGAAAACGTAACACMAATCTTCTAGTTTC—TTGTGTG 235
 * * * * * * * * * * * * * * * * *

M. pneumoniae —AA—TAAATACCAAAG—ATCAATAC—AATAAGTTACTAAGGGCTATGCT 252
M. genitalium —AAAATAAATACCAAAG—ATCAATAC—AATAAGTTACTAAGGGCTATGCT 224
M. pirum AATAACACAAATATCAAAATGCTAATGGATATCAAA—AATAAGTTACTAAGGGCTATGCT 295
 * * * * * * * * * * * * * * * * *

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FIG. 2a

<i>A. exanthum</i> ?	AAACAATTCTTCATTTGTCATCATATTGCGTTTTCGACAGCTT-----	88
<i>A. oculi</i> ?	AAACAATTCTCTAAATTTGTCATCATATTGCGTTTTCGACAGCTATGTCA-----	110
<i>A. laidiawii</i> ?	TAACATTCTCTAAATTTGTCATCATATTGCGTTTTCGACAGCTAAATGTCACTCAAAC	109
<i>A. laidiawii</i> ?	TAATATTCTCTAAATTTGTCATCATATTGCGTTTTCGACAGCTT-----	95
<i>A. modicum</i> ?	TTACAATCAATATACATTTCATCATATTGCGTTTTCGAGGATTTTCCTTC-----	78

* * * * * ***** * * *

FIG. 2b

<i>A. laidiawii</i> ?	CAAGTAACACATATTAATAATAAGTGGGCGCTGTAGCTCAGTTGGTATAGGDCACGCT	168
<i>A. oculi</i> ?	CAA-----AAGTGGGCGCTGTAGCTCAGTTGGTATAGGDCACGCT	156
<i>A. exanthum</i> ?	TAG-----TAATGGGCGCTGTAGCTCAGTTGGTATAGGDCACGCT	133
<i>A. modicum</i> ?	TTA-----TGGCGCTGTAGCTCAGCTGGTATAGGDCACGCT	122

<i>A. laidiawii</i> ?	TGATAAGCGTGGGCTCGATGGTTCAAGTCCATTGAGGCCACCATTAATAAATATCAATA	227
<i>A. oculi</i> ?	TGATAAGCGTGGGCTCGATGGTTCAAGTCCATTGAGGCCACCAT-----	201
<i>A. exanthum</i> ?	TGATAAGCGTGGGCTCGATGGTTCAAGTCCATTGAGGCCACCATTTATAT-----	184
<i>A. modicum</i> ?	TGATAAGCGTGGGCTCGATGGTTCAAGTCCATTGAGGCCACCATTATAG-----	172

***** * ***** * * *****

FIG. 2c

<i>A. laidiawii</i> ?	GTAA-TATTCTCTAAATTTGTCATCATATTTCAGTTTTCGAAAGACTTAA-AGTAATT-	104
<i>A. oculi</i> ?	GCAACAATTTCTCTAA-TTGTGTCATCATATTTCAGTTTTCGAAAGACTTAA-TCCAAGTG	115
<i>A. exanthum</i> ?	-AAACAATTTCTTCA-TTGTGTCATCATATTTCAGTTTTCGAAAGACTTTG-ACTTGT	98
<i>A. modicum</i> ?	-----CATTTGTCATCATATTTCAGTTTTCGAAAGACTTTTTCCTTCTAATAT	84

* ***** * * *

<i>A. laidiawii</i> ?	---TAAATGTTTCAAGAGTAAGAAAGTCTTTGAAAGTAGATAAAATGATGTCTGAAA-	180
<i>A. oculi</i> ?	A---TTGGTTTCTTAAAGTATCAAAATAAGTCTTTGAAAGTAGATAAATGATGTCTGAAA-	172
<i>A. exanthum</i> ?	C-TCAAG---AAGTATCAAAATAAGTCTTTGAAAGTAGATAAAAGAGTCTGAAAT	180
<i>A. modicum</i> ?	AAGTAAGTCTTTGAAAGTAGATAAATTCCTGTCTGTA-CAATAATATAAAAAGACAA	149

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FIG. 3a

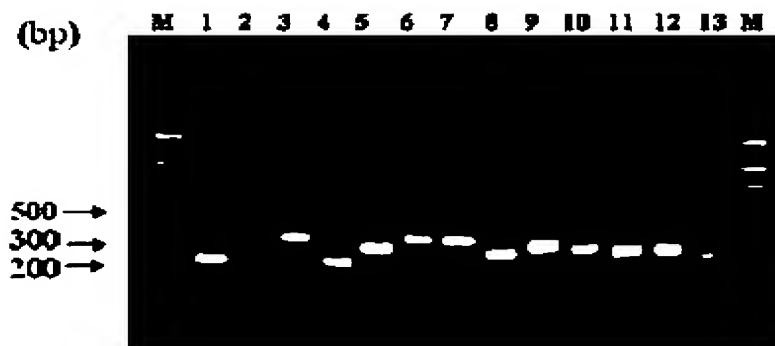


FIG. 3b

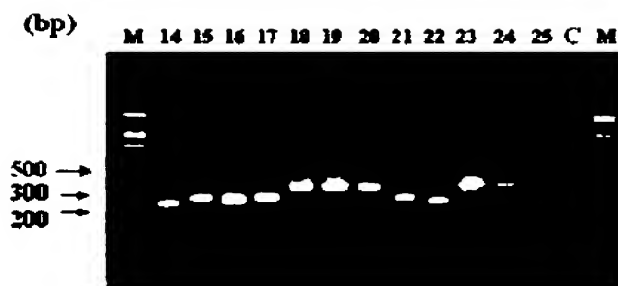


FIG. 4

MP-C [7]	<i>M. arginini</i> [28]	<i>M. orthorhoidis</i> [30]	<i>M. fermentans</i> [33]	<i>M. hominis</i> [38]	<i>M. hyorhinae</i> [41]
<i>M. neurolyticum</i> [49]	<i>M. opalescens</i> [52]	<i>M. orale</i> [58]	<i>M. pirum</i> [61]	<i>M. penetrans</i> [69]	<i>M. pulmonis</i> [75]
<i>M. salivarium</i> [83]	<i>M. cibacale</i> [85]	<i>M. faisonis</i> [87]	<i>M. faucium</i> [30]	<i>M. hyosynoviae</i> [90]	<i>M. muris</i> [92]
<i>M. primateum</i> [96]	<i>M. spermophilum</i> [100]	<i>M. synoviae</i> [105]	<i>M. pneumoniae</i> [110]	<i>M. genitalium</i> [114]	<i>M. bovis</i> [120]
<i>U. urealyticum</i> [122]			AP-C [22]	<i>A. laidlawii</i> [128]	MP-C [7]

*[] corresponds to SEQ ID No's of Tables 2 and 3.

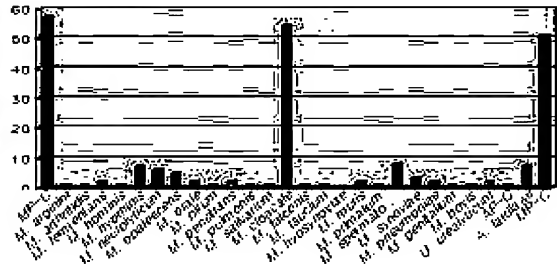


FIG. 5b

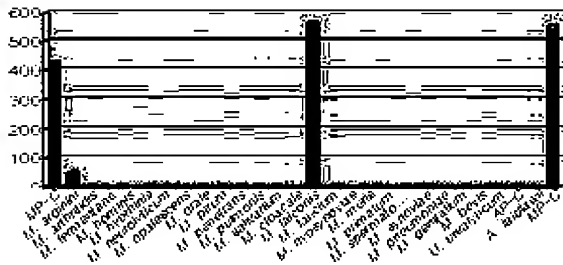
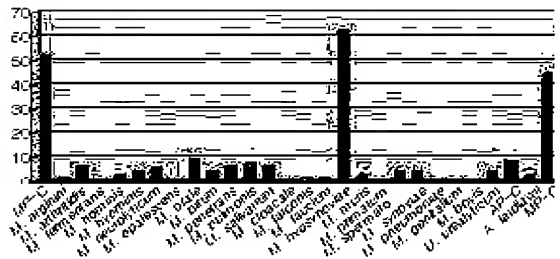
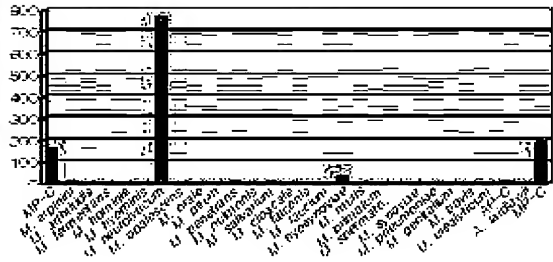
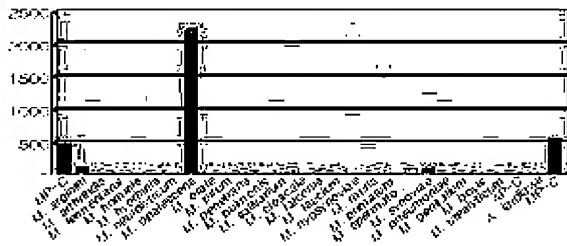


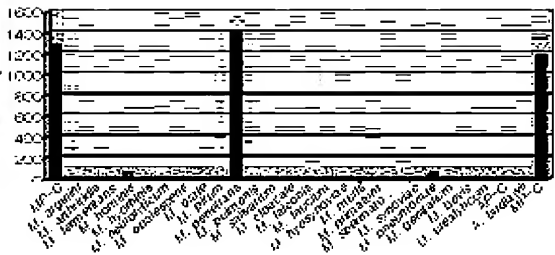
FIG. 5c

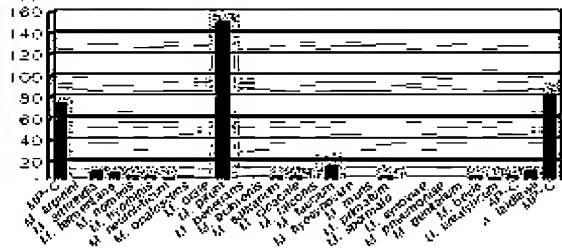


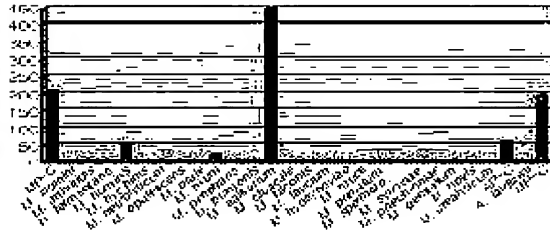




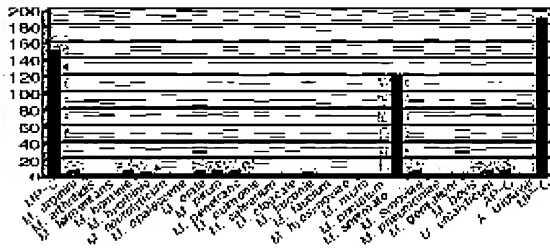
the 1990s, the number of people in the United States who are 65 years of age or older has increased by 50 percent. The number of people 75 years of age or older has increased by 100 percent. The number of people 85 years of age or older has increased by 200 percent. The number of people 95 years of age or older has increased by 400 percent. The number of people 100 years of age or older has increased by 800 percent. The number of people 105 years of age or older has increased by 1,600 percent. The number of people 110 years of age or older has increased by 3,200 percent. The number of people 115 years of age or older has increased by 6,400 percent. The number of people 120 years of age or older has increased by 12,800 percent. The number of people 125 years of age or older has increased by 25,600 percent. The number of people 130 years of age or older has increased by 51,200 percent. The number of people 135 years of age or older has increased by 102,400 percent. The number of people 140 years of age or older has increased by 204,800 percent. The number of people 145 years of age or older has increased by 409,600 percent. The number of people 150 years of age or older has increased by 819,200 percent. The number of people 155 years of age or older has increased by 1,638,400 percent. The number of people 160 years of age or older has increased by 3,276,800 percent. The number of people 165 years of age or older has increased by 6,553,600 percent. The number of people 170 years of age or older has increased by 13,107,200 percent. The number of people 175 years of age or older has increased by 26,214,400 percent. The number of people 180 years of age or older has increased by 52,428,800 percent. The number of people 185 years of age or older has increased by 104,857,600 percent. The number of people 190 years of age or older has increased by 209,715,200 percent. The number of people 195 years of age or older has increased by 419,430,400 percent. The number of people 200 years of age or older has increased by 838,860,800 percent. The number of people 205 years of age or older has increased by 1,677,721,600 percent. The number of people 210 years of age or older has increased by 3,355,443,200 percent. The number of people 215 years of age or older has increased by 6,710,886,400 percent. The number of people 220 years of age or older has increased by 13,421,772,800 percent. The number of people 225 years of age or older has increased by 26,843,545,600 percent. The number of people 230 years of age or older has increased by 53,687,091,200 percent. The number of people 235 years of age or older has increased by 107,374,182,400 percent. The number of people 240 years of age or older has increased by 214,748,364,800 percent. The number of people 245 years of age or older has increased by 429,496,729,600 percent. The number of people 250 years of age or older has increased by 858,993,459,200 percent. The number of people 255 years of age or older has increased by 1,717,986,918,400 percent. The number of people 260 years of age or older has increased by 3,435,973,836,800 percent. The number of people 265 years of age or older has increased by 6,871,947,673,600 percent. The number of people 270 years of age or older has increased by 13,743,895,347,200 percent. The number of people 275 years of age or older has increased by 27,487,790,694,400 percent. The number of people 280 years of age or older has increased by 54,975,581,388,800 percent. The number of people 285 years of age or older has increased by 109,951,162,777,600 percent. The number of people 290 years of age or older has increased by 219,902,325,555,200 percent. The number of people 295 years of age or older has increased by 439,804,651,110,400 percent. The number of people 300 years of age or older has increased by 879,609,302,220,800 percent. The number of people 305 years of age or older has increased by 1,759,218,604,441,600 percent. The number of people 310 years of age or older has increased by 3,518,437,208,883,200 percent. The number of people 315 years of age or older has increased by 7,036,874,417,766,400 percent. The number of people 320 years of age or older has increased by 14,073,748,835,532,800 percent. The number of people 325 years of age or older has increased by 28,147,497,671,065,600 percent. The number of people 330 years of age or older has increased by 56,294,995,342,131,200 percent. The number of people 335 years of age or older has increased by 112,589,990,684,262,400 percent. The number of people 340 years of age or older has increased by 225,179,981,368,524,800 percent. The number of people 345 years of age or older has increased by 450,359,962,737,049,600 percent. The number of people 350 years of age or older has increased by 900,719,925,474,099,200 percent. The number of people 355 years of age or older has increased by 1,801,439,850,948,198,400 percent. The number of people 360 years of age or older has increased by 3,602,879,701,896,396,800 percent. The number of people 365 years of age or older has increased by 7,205,759,403,792,793,600 percent. The number of people 370 years of age or older has increased by 14,411,518,807,585,587,200 percent. The number of people 375 years of age or older has increased by 28,823,037,615,171,174,400 percent. The number of people 380 years of age or older has increased by 57,646,075,230,342,348,800 percent. The number of people 385 years of age or older has increased by 115,292,150,460,684,697,600 percent. The number of people 390 years of age or older has increased by 230,584,300,921,369,395,200 percent. The number of people 395 years of age or older has increased by 461,168,601,842,738,790,400 percent. The number of people 400 years of age or older has increased by 922,337,203,685,477,580,800 percent. The number of people 405 years of age or older has increased by 1,844,674,407,370,955,161,600 percent. The number of people 410 years of age or older has increased by 3,689,348,814,741,910,323,200 percent. The number of people 415 years of age or older has increased by 7,378,697,629,483,820,646,400 percent. The number of people 420 years of age or older has increased by 14,757,395,258,967,641,292,800 percent. The number of people 425 years of age or older has increased by 29,514,790,517,935,282,585,600 percent. The number of people 430 years of age or older has increased by 59,029,581,035,870,565,171,200 percent. The number of people 435 years of age or older has increased by 118,059,162,071,741,130,342,400 percent. The number of people 440 years of age or older has increased by 236,118,324,143,482,260,684,800 percent. The number of people 445 years of age or older has increased by 472,236,648,286,964,521,369,600 percent. The number of people 450 years of age or older has increased by 944,473,296,573,929,042,739,200 percent. The number of people 455 years of age or older has increased by 1,888,946,593,147,858,085,478,400 percent. The number of people 460 years of age or older has increased by 3,777,893,186,295,716,170,956,800 percent. The number of people 465 years of age or older has increased by 7,555,786,372,591,432,341,913,600 percent. The number of people 470 years of age or older has increased by 15,111,572,745,182,864,683,827,200 percent. The number of people 475 years of age or older has increased by 30,223,145,490,365,729,367,654,400 percent. The number of people 480 years of age or older has increased by 60,446,290,980,731,458,735,308,800 percent. The number of people 485 years of age or older has increased by 120,892,581,961,462,917,470,617,600 percent. The number of people 490 years of age or older has increased by 241,785,163,922,925,834,941,235,200 percent. The number of people 495 years of age or older has increased by 483,570,327,845,851,669,882,470,400 percent. The number of people 500 years of age or older has increased by 967,140,655,691,703,339,764,940,800 percent. The number of people 505 years of age or older has increased by 1,934,281,311,383,406,679,529,881,600 percent. The number of people 510 years of age or older has increased by 3,868,562,622,766,813,359,059,763,200 percent. The number of people 515 years of age or older has increased by 7,737,125,245,533,626,718,119,526,400 percent. The number of people 520 years of age or older has increased by 15,474,250,491,067,253,436,239,052,800 percent. The number of people 525 years of age or older has increased by 30,948,500,982,134,506,872,478,105,600 percent. The number of people 530 years of age or older has increased by 61,897,001,964,269,013,744,956,211,200 percent. The number of people 535 years of age or older has increased by 123,794,003,928,538,027,489,912,422,400 percent. The number of people 540 years of age or older has increased by 247,588,007,857,076,054,979,824,844,800 percent. The number of people 545 years of age or older has increased by 495,176,015,714,152,109,959,649,689,600 percent. The number of people 550 years of age or older has increased by 990,352,031,428,304,219,919,299,379,200 percent. The number of people 555 years of age or older has increased by 1,980,704,062,856,608,439,838,598,758,400 percent. The number of people 560 years of age or older has increased by 3,961,408,125,713,216,879,677,197,516,800 percent. The number of people 565 years of age or older has increased by 7,922,816,251,426,433,759,354,395,033,600 percent. The number of people 570 years of age or older has increased by 15,845,632,502,852,867,518,708,790,067,200 percent. The number of people 575 years







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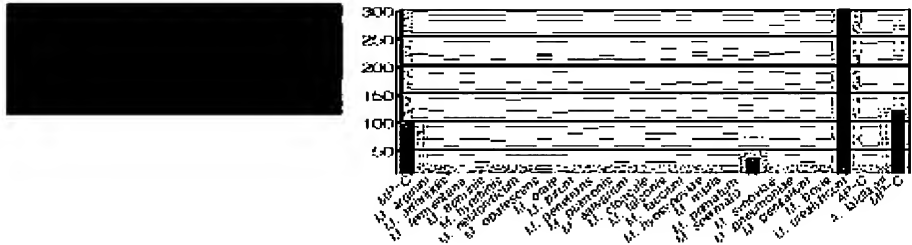


Figure 1: Bar chart showing the number of genes in each of the 20 clusters. The y-axis represents the number of genes, ranging from 0 to 1200. The x-axis lists the 20 clusters. Cluster 1 has the highest number of genes, exceeding 1200. Cluster 20 has the lowest number of genes, around 100. The chart shows a wide distribution of gene counts across the clusters.